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Amylase activity V.1

In 1 collection

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Protocol status: Working
We use this protocol and it's working

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Abstract

This protocol is a scaled-down, modified version of the Enzymatic Assay of α-Amylase (EC 3.2.1.1) found **here**.

Materials

Chemicals
Potato starch
D-(+)-maltose monohydrate
Sodium phosphate monobasic
Sodium chloride
potassium sodium tartrate, tetrahydrate
Sodium Hydroxide
3,5 Dinitrosalicylic acid

Equipment
Pipettes
- 2 -- 20 µL
- 20 -- 200 µL
- 100 -- 1000 µL

8-channel pipette (ca. 20 - 300 µL) [OPTIONAL, but recommended]
96 well microplate
Spectrophotometer (with 540 nm filter)

Protocol materials

1 M NaOH
1 M HCl
NaOH
Potassium sodium tartrate tetrahydrate **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**S2377

Before start

All instances of dH_2O can be replaced with ultrapure water.

Prepare solutions

15m

1 0.5% Potato starch solution

15m

1. add a small amount of dH_2O to a beaker
2. add [M] 0.5 Mass Percent 0.5% (w/v) potato starch
3. Boil for 00:15:00 while stirring
4. Take off heat and leave at room temperature
5. Add dH_2O to final volume
6. Continuous stirring throughout the assay

2 0.2% Maltose

1. add 100 mg maltose to 50 mL dH_2O

3 Buffer

1. add 250 mL dH_2O to a beaker
2. add 600 mg of sodium phosphate, monobasic
3. add 97.5 mg of sodium chloride
4. adjust to pH 6.9 with 1 M NaOH and 1 M HCl

4 2 M NaOH

1. add 0.8 g NaOH to 10 mL dH_2O

5 5.3 M potassium sodium tartrate, tetrahydrate solution

1. add 14.96 mg
   Potassium sodium tartrate tetrahydrate **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**S2377
   to 10 mL of the **2 M NaOH** solution
2. dissolve solids with heat and stirring

Safety information

DO NOT heat to a boil

6 **96 mM 3,5-Dinitrosalicylic acid solution**

1. add 0.438 g **3,5-dinitrosalicylic acid** to 20 mL **dH_2O**
2. dissolve solids with heat and stirring

Safety information

DO NOT heat to a boil

7 **Colour reagent**

1. add 12 mL of 50-70 °C **dH_2O** to an appropriate size amber bottle (or something that can protect the solution from light).
2. add (slowly and with mixing) 8 mL of warm [M] 5.3 Mass Percent
   Potassium sodium tartrate tetrahydrate **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**S2377
3. add 20 mL of warm **96 mM 3,5-Dinitrosalicylic acid solution**

Note

The colour reagent is stable for 6 months in a dark place at ambient temperature

Saliva collection

30s

8 **Saliva samples are included as a positive control for amylase activity.** 30s

Saliva donors rinse their mouth with water for 00:00:30

9 Collect the saliva by spitting into 50 ml plastic centrifuge tubes.

10 Centrifuge the saliva at 1000 x g, 00:10:00 and sample from the supernatant. 10m

Standard curve preparation

11 Two standard curves are prepared: one containing dH_2O, and one containing artificial saliva.

Protocol

NAME

Artificial saliva

CREATED BY

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Preview

11.1 Add 300 mL distilled (or deionized) dH_2O to a 1000 mL beaker, with stirring and heat 60 °C .

11.2 Add:

- 2.5 g

Mucin from porcine stomach (Type III) **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**M1778

- 5 g Trypticase™ Peptone **Thermo Fisher Catalog #**211921

- 10 g Oxoid™ Proteose Peptone **Thermo Fisher Catalog #**LP0085B
- 5 g Bacto Yeast Extract **Becton Dickinson (BD)**

Let the reagents completely dissolve before continuing to the next step

11.3 Add:

- 2.5 g KCl
- 0.35 g NaCl
- 0.2 g CaCl2
- 0.74 g
  Sodium phosphate dibasic **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**7558-79-4
- 0.54 g NaHCO3
- 2.5 mg Hemin

11.4 Add the remaining 700 mL distilled (or deionized) dH_2O

11.5 Adjust to pH 7 with NaOH and stirring

11.6 Transfer to two 1000 ml bottles, so half of each bottle is filled. 15m

Autoclave at 121 °C , 1 Bar for 00:15:00 minutes

Safety information

Do NOT screw bottle caps on tightly.

Loosely screw the caps on the bottles or cover the tops with foil

11.7 Once the solution has cooled, add:

- 1 mg Menadione
- 0.3 g Urea

- 0.17 g L-Arginine **Catalog #**A5006

11.8 Store in fridge at ca. 4 °C

Occasionally test the pH to ensure it stays around pH 7

12 Add the following reagents to the wells of a deep-well microplate so each well contains a total volume of 225 µL. Each column in the table represents a single well.

Add the Maltose to each well first, then dH_2O, then colour reagent.

	Reagent	STD1	STD2	STD3	STD4	STD5	STD6	STD7	STD Blank
	0.2% Maltose	3.75	15	30	45	60	75	150	0
	dH2O	146.25	135	120	105	90	75	0	150
	Colour reagent	75	75	75	75	75	75	75	75

All quantities are in µL

13 go to step #12 and repeat the process, but adding artificial saliva instead of dH_2O.

14 Boil the deep-well microplate(s) for 00:03:00 , then cool on ice to room temperature. 3m

15 Add 675 µL of dH_2O to each well for a final volume of 900 µL .

Sample preparation

16 Samples are prepared in a 96 deepwell plate with approx. 1 mL volume per well.

Samples should be analysed in duplicates or triplicates.

17 To each well, add 75 µL of sample (or saliva)

Note

It is best to make duplicates or triplicates of the samples

18 Then add 75 µL of the **0.5% potato starch solution** to each of the wells with samples (and saliva)

Safety information

Once the starch has been added to the sample, the reaction will begin. The starch should be added quickly; ideally with a multichannel pipette .

19 Incubate the plate at 36 °C for 00:06:00 6m

20 Remove the plate from the incubator and add 75 µL of colour reagent to the sample wells, 3m
then boil for 00:03:00 .

Safety information

The colour reagent will stop the reaction, so should be added with a multichannel pipette.

21 Cool the plate on ice to Room temperature

then add 675 µL **dH_2O** to each well for a final volume of 900 µL (homogenise the solution with the pipette).

Note

Use a new pipette tip for each sample.

22 then transfer 200 µL to a 96 well microplate suitable for the photometer.

Photometer

23

Equipment

Multiskan FC	NAME
Microplate Photometer	TYPE
Thermo Scientific	BRAND
51119000	SKU

Photometer settings:
540 nm filter

1. Photometer reading 1
2. Pause
3. Shake
4. Pause
5. Photometer reading 2

Note

Make sure to include a sample blank (with dH_2O for saliva positive controls, and stock artificial saliva for samples)

Calculations

24 Calculate ΔA_{540} of each Standard by subtracting the Standard Blank.

$$\Delta A_{540\ Standard} = \Delta A_{540\ Standard} - \Delta A_{540\ StandardBlank}$$

25 Prepare the standard curve by regressing (OLS) the ΔA_{540} of each Standard on mg Maltose (go to step #12)

26 Calculate ΔA_{540} of each Sample by subtracting the Sample Blank.

$$\Delta A_{540\ Sample} = \Delta A_{540\ Sample} - \Delta A_{540\ SampleBlank}$$

27 Then calculate the mg of Maltose released (x) using the regression coefficients:

$$\frac{y - b}{a} = x$$

Where *y* is the $\Delta A_{540\ Sample}$, *b* is the intercept, and *a* is the slope.

28 Units per mL enzyme can be calculated as:

$$U/mL\ enzyme = \frac{mg\ Maltose\ released \times dilution\ factor}{mL\ enzyme}$$

where *dilution factor* is how much the sample was diluted (if at all), and *mL enzyme* is how much of the sample was added in step 17. go to step #17

A *Unit* is defined as mg of maltose released from starch in 6 minutes.